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EXAMINER	
CANELLA, KAREN A	
ART UNIT	**PAPER NUMBER**
1643	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/801,471	TERSTAPPEN ET AL.	
	Examiner	Art Unit	
	Karen A. Canella	1643	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☐ Claim(s) 1-4,6-14,16-23,27-31,33-42,46-50 and 54 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) 1-4,6-14,16-23,27-31,33-42,46-50 and 54 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Claims 5, 15 and 32 have been canceled. Claims 1, 7, 10, 16, 20, 28, 35, 37 and 47 have been amended. Claims 1-4, 6-14, 16-23, 27-31, 33-42, 46-50 and 54 are pending and under consideration. The finality of the Office action of March 11, 2003 is withdrawn in light of the new grounds of rejection below.

Sections of Title 35, U.S. Code not found in this action can be found in a prior action.

Applicant is reminded that the non-provisional and provisional applications upon which a priority claim is based (09/248,388, 60/074,535, 60/110,279 and 60/110,202) fail to provide an adequate written description of the invention because none of the documents make mention with the stabilized cells of the instant invention and therefore the priority date of the instant application is March 7, 2001.

Claims 1-4, 6-14, 16-23, 27-31, 33-42, 46-50 and 54 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.
Claim 47 recites and "improved" kit but fails to specify what the improvement actually comprises.

(A) Claims 6 and 33 are vague and indefinite for depending upon a canceled claim.

(B) Claims 1, 7, 10, 20, 37 recite "two distinct fluorescent labels having the same spectral properties". It is unclear how distinct fluorescent labels can have the same spectral properties because alteration of the structure of the fluorophore to satisfy the limitation of "distinct" would result in an alteration in the spectral excitation and/or emission properties of said fluorphore relative to the original fluorophore. For purpose of examination, the "two distinct fluorescent labels having the same spectral properties" will be read as having the same fluorophore in order to comply with the limitation of "having the same spectral properties" but the distinctness will be attributed to the agent which would carry the label, such as antibodies targeted to two distinct antigens, probes which target two distinct cellular structures or nucleic acids which target two different genes for in situ hybridization.

(C)The recitation of "membrane dye" in claim 9 lacks antecedent basis in claim 7.

(D)The recitation of "cell fixative" rather than "fixative" in claims 12 and 18 lacks antecedent basis in claims 11 and 17, respectively.

(E)Claims 11 and 17 are vague and indefinite because it is unclear how the buoyant density medium in which the cell of claim 10 or claim 16 is suspended further limits the stabilized cell of claim 10 or claim 16, respectively.

Claims 1, 2, 4, 10, 12, 14, 28, 29 and 31 are rejected under 35 U.S.C. 103(a) as being unpatentable over Davis (EP 469,766, reference B1 of the IDS filed Sep 25, 2001) as evidenced by Leif et al (US 5,188,935, cited in a previous action) in view of Okada et al (U.S. 6,753,190).

Claim 1 is drawn to a process for producing a stabilized cell, said process comprising: the redundant labeling of said control cell with at least two distinct florescent labels having the same spectral properties; contacting said labeled cells with a fixative; removing excess fixative to promote long-term storage, said control cells being biologically stable for a period up to at least six months. Claim 2 specifies the fixatives of paraformaldehyde, formaldehyde and glutaraldehyde and glyoxal. Claim 4 specifies that the label is an antibody immunologically specific for an antigen present on said cells, said antibody having a fluorescent conjugate. Claim 10 is drawn to a stabilized cell, wherein said control cell is labeled redundantly with at least two distinct fluorescent labels having the same spectral properties and cellular components and antigenic moieties of said control cell have been stabilized for a period up to at least six months by exposure to fixative. Claim 12 specifies the fixatives of paraformaldehyde, formaldehyde and glutaraldehyde and glyoxal. Claim 14 specifies said control cells immunologically specific for an antigen present on said cells, said antibody having a fluorescent conjugate. Claim 28 is drawn in part to a stabilized cell comprising a redundantly labeled membrane, said membrane being labeled with at least two fluorescent labels having the same spectral properties , said cells further comprising stabilized cellular components and antigenic moieties , said stabilization being affected by exposure to fixative, wherein said control cells are tumor cells. Claim 29 specifies the fixatives of paraformaldehyde, formaldehyde and glutaraldehyde and glyoxal. Claim 31 specifies said control cells immunologically specific for an antigen present on said cells, said antibody having a fluorescent conjugate.

Davis teaches a method of making a control cell which is fixed by paraformaldehyde, said excess paraformaldehyde being removed after fixing (column 9, lines 20-30). Davis teaches that cells so fixed retain their ability to be tagged with antibodies conjugated to fluorescent markers, stains such as fluorescein, rhodamine and cyanine dyes and nucleic acid stains (column 5 , line 39 to column 6, line 1). Davis et al teach that said control cells may be derived for leukemias, cancers (column 5, lines 16-20, column 8, lines 4-6) and tumor cell lines (column 8, lines 8-11). Davis teaches that the cells may be dried after fixation but before labeling or conversely may be labeled after fixation but prior to drying. Davis does not specifically teach that the fixed cells or the fixed and dried cells would be biologically stabilized for six months, however, the method of paraformaldehyde fixation appears to be identical to the claimed method of fixation, therefore it is reasonable to assume that the fixed cells of Davis would be biologically stable for at least six months. Davis does not specifically teach the order of the claimed method steps wherein the cells are labeled before being fixed (column 8, lines 45-49). Leif et al teach that antigen-antibody complexes on leukocytes are preserved after cross-linking with dialdehyde fixing reagents (column 4, lines 4-16). Therefore, it would be reasonable to assume that the control cell of Davis could be labeled before or after fixing with glutaraldehyde or glyoxal. Lief et al do not teach the redundant labeling of the fixed cells, the stability of the fixed cells, nor the labeling of tumor cells or tumor cell lines for use as control cells. Davis does not specifically teach the labeling of the cells with at least two distinct fluorescent labels having the same spectral properties.

Okada et al teach first and second labeled substances which constitute first and second labeled immunochemical components. Okada et al teach that the capturing of the double labeled immunological complexes by the analytes constitutes an amplification of the detection signal, making it possible to detect said analyte at a higher sensitivity because the labeled substances are assembled at one place and bound to amplify a detection signal making it possible to detect the presence of the analyte at a higher sensitivity (column 6, lines 34-40).

It would have been prima facie obvious at the time the claimed invention was made to use two antibodies that would react with two distinct antigens on the fixed cell of Davis et al, wherein each antibody were conjugated to the same detectable moiety, such as the same fluorphore. One of skill in the art would have been motivated to do so by the teachings of Okada

et al on the advantages of causing an analyte to capture two different immunological reagents such that each of the reagents were then assembled at one place (on the surface of the cell) making it possible to detect the signal of the analyte at higher sensitivity. One of skill in the art would conclude that the amplification of the signal was the result of the additive emission from each of the labeling moieties wherein the spectral characteristics were additive.

Claims 35 and 36 are rejected under 35 U.S.C. 103(a) as being unpatentable over Griwatz et al (Journal of Immunological Methods, 1995, Vol. 183, pp. 251-265) in view of Maples (WO 94/16314, cited in a previous action).

Claim 35 is drawn to an improved method of detecting an enumerating rare cells in a mixed cell population, the presence of said cells being indicative of severity of a disease state comprising: (a) obtaining a blood sample suspected of containing said rare cells; (b) preparing an immunomagnetic sample wherein said biological specimen is mixed with magnetic particles coupled to a ligand which reacts specifically with a determinant of the rare cells; (c) contacting said immunomagnetic sample with at least one reagent which labels a determinant of said rare cells, and (d) analyzing the labeled rare cells to determine the presence and number of any rare cells, wherein the greater the number of rare cells present in said sample, the greater the severity of the disease, wherein the improvement comprises the addition of a stabilized cell for use as an internal control in said method, said control cell having determinants in common with said rare cells, and wherein said membrane of said control cell is detectably labeled and cellular components and antigenic moieties of said control cells have been stabilized for a period up to six months by exposure to fixative. Claim 36 specifically embodies the method of claim 35 wherein said rare cell is a cancer cell and said disease is cancer.

Griwatz et al teach a method of making a stabilized tumor cell comprising harvesting spiked MCF-7 cells from a gradient band (page 253, first column, lines 12-17 under the heading of section 2.3) and fixing and permeablizing said cells (page 253, under the heading of section 2.4) followed by preparing an immunomagnetic sample wherein said biological specimen is mixed with magnetic particles coupled to an anticytokeratin 8/18 antibody (page 253, under the section 2.5) which reacts specifically with cytokeratins in epithelial cells rather than with hematopoietic cells in the peripheral blood.. Griwatz et al teach a similar procedure using

immunomagnetic particles bound to prostatic epithelial cells, wherein said prostatic cells were counterstained with anti-PSA antibody (page 255, first column, under section 2.12) which fulfills the specific embodiment of section c of claim 35 "contacting said immunomagnetic sample with at least one reagent which labels a determinant of said rare cells".. Griwatz et al do not specifically teach the addition of a the MCF-7 fixed and stabilized cell, or the prostatic tumor fixed and stabilized cells for use as an internal control in said method, wherein said control cell is detectably labeled with at least two fluorescent labels having the same spectral properties and cellular components and antigenic moieties of said control cells have been stabilized for a period up to six months by exposure to fixative, however the paraformaldehyde is used by Griwatz et al to fix and stabilize the MCF-7 cells, as is taught by the instant specification to stabilize cells for a period of up to six months, therefore one of skill in the art would reasonably conclude that the fixed stabilized MCF-7 or prostatic tumor cells would be stabile for up to six months.

Maples et al teach that the use of labeled preserved cells represents an improvement in the art over the use of fluorescent beads as said cells have the same size shape and light scatter characteristics of the analyte (page 3, lines 8-31).

It would have been prima facie obvious at the time the invention was made to used the fixed and permeablized MCF7 cells or prostate epithelial cells, conjugated to immunomagnetic particles as taught by Griwatz et al in a method of detecting an enumerating rare cells in a mixed cell population, comprising isolating a single band in a density gradient of peripheral blood cells from a patient, fixing and stabilizing the cells therein, and attaching said cells to immunomagnetic particles by means of reacting said cells with FITC conjugated anti-human cytokeratin 8/18 which is conjugated to immunomagnetic beads, wherein the rare cells were circulating epithelial cancer cells and the mixed population the peripheral blood of a patient. One of skill in the art would have been motivated to carry out the method by the suggestion of Griwatz et al that cancer cells can be enriched from peripheral blood at an enrichment factor which ensures the capture of migrating cancer cells in the peripheral blood, and permits the isolation of enough cancer cells to submit said cells to further molecular and genetic analysis (page 262, second column, lines 6-13). One f skill in the art would have been motivated to use the MCF-7 fixed and stabilized and conjugated to the immunomagnetic particle as a control cell

in the actual analysis of the peripheral blood of patients because of the teachings of Maples et al on the superiority of cells over that of fluorescent beads in assays involving cells.

Claims 35, 36 and 38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Griwatz et al (Journal of Immunological Methods, 1995, Vol. 183, pp. 251-265) and Maples (WO 94/16314) as applied to claims 35 and 36 above, and further in view of Waggoner et al (Human Pathology, 1996, Vol. 27, pp. 494-502, cited in a previous action) and Haugland (Handbook of Fluorescent Probes and Research Chemicals, 1992, 5th Edition, pp. 235-269, cited in a previous action).

Claim 38 specifically embodies the method of claim 35 wherein said membrane label is selected from the group consisting of long chain lipophillic indocarbocyanines, indocarbocyanines, indodicarbocyanines and analogs thereof, aminostyrl dyes, C18 rhodamine B, and C18 fluorescein dyes.

The combination of Griwatz et al and Maples renders obvious the specific embodiment of claims 35 and 36. Griwatz et al teach that the advantages of targeting intracytoplasmic proteins for labeling is that they are not cleaved by pericellular proteases, are not major immune response entities, and their expression is highly conserved and less variable (page 263, first column bridging paragraph to second column). Griwatz et al do not specifically teach the targeting of lipophillic proteins integral to the cell membrane.

Waggoner et al teach the use of diL-C18 as a membrane label to establish the location of the cellular membrane during fluorescence imaging (Table 1, "Membrane location and fluidity"). Dil-C18 is defined as an analog of carboindocyanines as stated on page 25, line 1 of the instant specification.

Haugland teaches the octadecyl ester of fluorescein (page 253, under the heading "Lipophilic Fluorescein Probes"), the octadecyl indocarbocyanines and oxacarbocyanines and analogs thereof, dialkyamnostyrl and octadecyl rhodamine B (pages 260-261), all of which have the property of staining membranes.

It would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made to substitute the membrane stains taught by Waggoner et al and Haugland for one or more of the antibodies which bind to distinct antigens on the surface of the

cell. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Griwatz et al on the advantages of targeting cellular molecules which are not cleaved by proteases, wherein said targets are highly conserved and less variable than surface antigen-directed antibodies.

Claims 1-4, 10-14, 28-31 and 34-38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Griwatz et al and Maples and Waggoner et al and Haugland. as applied to claims 35, 36 and 38 above, and further in view of Okada et al (U.S. 6,753,190).

The specific embodiments of claims 1, 2, 4, 10, 12, 14, 28, 29, 31, 35, 36 and 38 are set forth above. Claims 11 and 34 embody the control cell of claim 1 and claim 28, respectively, suspended in a buoyant density medium. Claims 3, 14, 30 and 38 embody the methods of claims 1 and 35 and the cells of claims 10, 28, wherein the labels are membrane labels selected from the group consisting of long chain lipophilic carbocyanines, long chain lipophilic indocarbocyanines, long chain lipophilic indodicarbocyanines, lipophilic aminostyryl dyes, and long chain analogs of C18 rhodamine B and C18 fluorescein dye. Claim 37 specifically embodies the method of claim 35 wherein said membrane is redundantly labeled with at least two fluorescent labels having the same spectral properties.

The combination of Griwatz et al, Maples, Waggoner et al and Haugland render obvious the limitations of claims 35, 36 and 38 for the reasons set forth above. None of the reference teach the redundant label of the control cell with at least two distinct fluorescent labels having the same spectral properties.

Okada et al teach first and second labeled substances which constitute first and second labeled immunochemical components. Okada et al teach that the capturing of the double labeled immunological complexes by the analytes constitutes an amplification of the detection signal, making it possible to detect said analyte at a higher sensitivity because the labeled substances are assembled at one place and bound to amplify a detection signal making it possible to detect the presence of the analyte at a higher sensitivity (column 6, lines 34-40).

It would have been prima facie obvious at the time the claimed invention was made to use two antibodies that would react with two distinct antigens on the fixed cell of Griwatz et al, wherein each antibody were conjugated to the same detectable moiety, such as the same

fluorphore. One of skill in the art would have been motivated to do so by the teachings of Okada et al on the advantages of causing an analyte to capture two different immunological reagents such that each of the reagents were then assembled at one place (on the surface of the cell) making it possible to detect the signal of the analyte at higher sensitivity. One of skill in the art would conclude that the amplification of the signal was the result of the additive emission from each of the labeling moieties wherein the spectral characteristics were additive. Further, it would have been prima facie obvious to use the membrane labels taught by Waggoner et al and Haugland, as one of the targeted cellular structures, wherein the fluorescent portion of the probe would have the same spectral characteristics as a labeled antibody targeting a cell surface antigen. One of skill in the art would have been motivated to do this by the teachings of Griwatz on the desirability of cellular structures which are not cleaved by pericellular proteases or recognized as immune responsive entities (page 263, bottom of column 1 to top of column 2). One of skill in the art would be motivated to have both labeling agents which target distinct cellular structures but have the same spectral characteristics in order to detect the labeled cells at a higher sensitivity.

Claims 16-18 and 47 are rejected under 35 U.S.C. 103(a) as being unpatentable over Terstappen et al (WO 99/41613) in view of Davis et al (EP 469,766, reference B1 of the IDS filed Sep 25, 2001) and Maples et al (WO 94/16314).

Claim 16 is drawn to a stabilized cell having determinants in common with rare cells, said stabilized cell comprising a detectably labeled membrane and stabilized cellular components and antigenic moieties due to exposure to fixative, wherein said control cell is a tumor cell expressing EpCAM on its surface and intracellular cytokeratin. Claim 17 specifically embodies the control cell of claim 16, said control cell suspended in a buoyant density medium. Claim 18 specifically embodies the cell of claim 16 wherein said fixative is selected from the group consisting of paraformaldehyde, formaldehyde and glutaraldehyde and glyoxal. Claim 47 is drawn to an improved kit for the screening of a patient for the presence of circulating tumor cells comprising: (a) coated magnetic nanoparticles comprising a magnetic core material, a protein base coating material and anti-EpCAM coupled directly or indirectly to said base coating material; (b) at least one antibody having a binding specificity for a cancer cell determinant; (c)

cell specific dye for excluding sample components for other than said tumor cells; wherein the improvement comprises the addition of a container comprising stabilized cells for use in an internal control, said stabilized control cells having determinants in common with said rare cells, wherein said membrane of said control cell is detectably labeled and cellular components and antigenic moieties of said control cells have been stabilized for up to at least six months by exposure to fixative, said stabilized control cells being suspended in buoyant density medium.

Terstappen et al teach a method for detecting an enumerating rare cells in a mixed cell population, especially circulating tumor cells. Terstappen teaches the use of a kit comprising coated, magnetic nanoparticles coupled, directly or indirectly to a biospecific ligand that has affinity for a first characteristic determinant on a cancer cell; ii. at least one biospecific reagent having binding specificity for a second characteristic determinant present on a cancer cell; and iii. a cell specific dye for excluding non-target entities from analysis, wherein the kit include an antibody having affinity for a tumor cell marker, colloidal magnetic particles are conjugated to anti-EpCAM (an antibody having binding specificity for epithelial cell adhesion molecule), the biospecific reagents comprise a panel of monoclonal antibodies and the cell specific dye stains nucleic acids. Terstappen et al teach that other enrichment means for the rare cells, such as density gradient centrifugation may be used in conjunction with the immunomagnetic separation. Terstappen et al do not teach the use of a preserved stabilized control cell in said method.

Davis teaches a method of making a control cell which is fixed by paraformaldehyde, said excess paraformaldehyde being removed after fixing (column 9, lines 20-30). Davis teaches that cells so fixed retain their ability to be tagged with antibodies conjugated to fluorescent markers, stains such as fluorescein, rhodamine and cyanine dyes and nucleic acid stains (column 5 , line 39 to column 6, line 1). Davis et al teach that said control cells may be derived for leukemias, cancers (column 5, lines 16-20, column 8, lines 4-6) and tumor cell lines (column 8, lines 8-11). Davis teaches that the cells may be dried after fixation but before labeling or conversely may be labeled after fixation but prior to drying. Davis does not specifically teach that the fixed cells or the fixed and dried cells would be biologically stabilized for six months, however, the method of paraformaldehyde fixation appears to be identical to the claimed method of fixation, therefore it is reasonable to assume that the fixed cells of Davis would be biologically stable for at least six months. Leif et al teach that antigen-antibody complexes on

leukocytes are preserved after cross-linking with dialdehyde fixing reagents (column 4, lines 4-16). Therefore, it would be reasonable to assume that the control cell of Davis could be labeled before or after fixing with glutaraldehyde or glyoxal.

Maples et al teach that the use of labeled preserved cells represents an improvement in the art over the use of fluorescent beads as said cells have the same size shape and light scatter characteristics of the analyte (page 3, lines 8-31).

It would have been prima facie obvious at the time the claimed invention was made to provide a stabilized control cell as part of the method an kit taught by Terstappen et a. One of skill in the art would have been motivated to so by the teachings of Maples on the improvement afforded by the use of a labeled preserved cell over that of a fluorescent bead, and by the teachings of Davis et al on how to make a control cell which is stabilized.

Claims 16-18, 21, 22 and 47-49 are rejected under 35 U.S.C. 103(a) as being unpatentable over Terstappen et al (WO 99/41613) and Davis et al (EP 469,766) and Maples et al (WO 94/16314) as applied to claims 16-18 and 47 above, and further in view of Griwatz et al (Journal of Immunological Methods, 1995, Vol. 183, pp. 251-265) Brandes et al (Cancer Research, 1983 Vol. 6, pp. 2831-2835) and Hudziack et al (U.S. 5,677,171).

Claim 48 embodies the kit of claim 47 wherein the control cell is a SKBR3 breast cancer cell further comprising a second detectably labeled surface determinant selected from the group consisting of mammoglobulin, human milk fat globulin, and Her-2/neu. Claim 49 embodies the kit of claim 47 wherein the control cell a MCF-7 cell further comprising a second detectably labeled surface determinant which is an estrogen receptor.

Terstappen et al teach the kit comprising a panel of antibodies which specifically bind to a cancer antigen which includes an anti-Her-2 antibody and an anti-estrogen receptor antibody.

Griwatz et al teach a stabilized control cell comprising MCF-7 or SKBR3. Griwatz et al do no teach the further labeling of each cell with an anti-estrogen antibody or an anti-Her-2 antibody.

Brandes et al (Cancer Res. 1983 Jun;43(6):2831-5) teach that the MCF-7 cell line is an estrogen receptor positive cell line.

Hudziack et al (U.S. 5,677,171) teach that the breast tumor cell line of SK-BR-3 expresses the Her-2 receptor.

It would have been prima facie obvious at the time the claimed invention was made to make control cells for breast cancer detection, wherein said cell was either MCF-7 carrying a label for the estrogen receptor, or SKBR3 carrying a label for the Her-2 receptor. One of skill in the art would have been motivated to provide such control cells in order to facilitate the detection of circulating breast cancer cells which are estrogen receptor or her-2 positive.

Claims 16-18, 23, 47 and 50 are rejected under 35 U.S.C. 103(a) as being unpatentable over Terstappen et al WO 99/41613 and Davis et al and Maples et al as applied to claims 16-18 and 47 above, and further in view of Griwatz et al and Boffa et al (Journal of Biological Chemistry 1996, Vol. 271, pp.13228-13233).

Claim 50 embodies the kit of claim 47 wherein the control cell is a LNCaP prostate cancer cell further comprising a second detectably labeled surface determinant selected from the group consisting of PSMA, PSA and androgen receptor

Terstappen et al teach the kit comprising a panel of antibodies which specifically bind to a cancer antigen which includes an anti-androgen receptor antibody.

Griwatz et al teach a stabilized control cell comprising MCF-7 or SKBR3.

Boffa et al teach that prostate cell line LNCap is an androgen receptor positive cell line.

It would have been prima facie obvious at the time the claimed invention was made to use a control cell comprising a LNCaP prostate cancer cell that was detectably labeled on the androgen receptor. One of skill in the art would have been motivated to do so by the teachings of Griwatz on using the breast cancer cell lines of MCF-7 or SKBR3 for making stabilized control cells, and the suggestion of Terstappen et al that a panel of antibodies which specifically bind to the androgen receptor be part of the invention. One of skill in the art would be motivated to use a stabilized LNCaP cell which was carrying a detectable label on the androgen receptor for the detection of circulating prostate cancer cells which were androgen positive.

Claims 16-18, 27, 47 and 54 are rejected under 35 U.S.C. 103(a) as being unpatentable over Terstappen et al WO 99/41613 and Davis et al and Maples et al as applied to claims 16-18 and

47 above, and further in view of Griwatz et al (Journal of Immunological Methods, 1995, Vol. 183, pp. 251-265), Shih et al (Cancer Research, 1994, Vol. 54, pp. 2514-2520), Shih (Journal of Pathology, 1999, Vol. 189, pp. 4-11) and the abstract of Silverman.

Claim 27 embodies the control cell of claim 16, said cell being a C32 melanoma cell further comprising a second detectably labeled surface determinant which is a CD146 molecule.

Claim 54 embodies the kit of claim 47 wherein the control cell is a C32 melanoma cell further comprising a second detectably labeled determinant which is CD146.

Terstappen et al teach that the disclosed methods are useful in the detection of melanoma cells (page 75, lines 8-10 and 17). Terstappen et al do not teach C32 control cells comprising a detectable label on the CD146 molecule.

Shih (1999) teaches that the detection of CD146 is useful in the diagnosis of melanoma (abstract) and that a synonym for the CD146 antigen is the A32 antigen (page 4, second column, lines 1-6).

Shih et al (1994) teach that the A32 antigen is expressed on the cell surface of most melanomas and on melanoma cell lines (abstract and Figure 7 on page 2519). The abstract of Silverstein et al teaches that C32 cells are wild-type melanoma cells. It would be reasonable to conclude that C32 cells expressed the CD146/A32 antigen.

Neither the abstract of Shih, Shih (1994) or Silverstein et al teach fixed stabilized C32 cells comprising a detectably labeled CD146 molecule.

It would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention to use stabilized C32 cells having a detectably labeled cell surface determinant which is a CD146 molecule to aid in the detection of circulating melanoma cells. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of the abstract of Silverstein on C32 cells being representative of melanoma and the teachings of Shih et al on the CD146/A32 molecule as being diagnostic for melanoma and the presence of the CD146/A32 antigen on melanoma cell lines..

Claims 1, 2, 4, 7, 8, 10, 16-18, 20, 28, 29, 31, 34, 35, 36, 37, 39 and 47 rejected under 35 U.S.C. 103(a) as being unpatentable over Terstappen et al and Davis et al and Maples et al as applied to claims 16-18 and 47 above, and further in view of Okada (U.S. 6,753,190).

Claim 7 is drawn to a process for producing a stabilized cell for use an internal control in methods for isolating and identifying rare cell comprising redundantly labeling said control cells with at least two distinct fluorescent labels having the same spectral properties; permeabilizing said cell; contacting the labeled cells with a cell fixative, said fixative effecting stabilization of both cellular structure and antigenic moieties present on said control cells; subsequently removing the excess fixative to promote said long-term storage of control cells, said control cells being physically and biologically stable for at least six months, wherein said control cell expresses epithelial cell adhesion molecule on its surface and also expresses cytokeratin. Claim 8 embodies the method of claim 7 wherein said cell fixative is selected from the group consisting of paraformaldehyde, formaldehyde, glutaraldehyde and glyoxal.

The combination of Terstappen et al, Davis et al and Maples et al renders obvious the use of a control stabilized cell coupled to magnetic particles via an anti-EpCAM antibodies. The combination does not teach that said control cell is redundantly labeled with at least two fluorescent labels.

Okada et al teach first and second labeled substances which constitute first and second labeled immunochemical components. Okada et al teach that the capturing of the double labeled immunological complexes by the analytes constitutes an amplification of the detection signal, making it possible to detect said analyte at a higher sensitivity because the labeled substances are assembled at one place and bound to amplify a detection signal making it possible to detect the presence of the analyte at a higher sensitivity (column 6, lines 34-40).

It would have been prima facie obvious to redundantly label said control cell with binding agents that bind to different moieties on the cell but which are conjugated to the same fluorescent moiety. One of skill in the art would have been motivated to do so by the teachings of Okada et al on the higher sensitivity afforded by concentration of a detectable signal on a single cell.

Claims 16-19 and 47are rejected under 35 U.S.C. 103(a) as being unpatentable over Terstappen et al (WO 99/41613), Davis et al (EP 469,766) and Maples et al (WO 94/16314) as applied to claims 16-18 and 47 above, and in further view of Griwatz et al (Journal of Immunological Methods, 1995, Vol. 183, pp. 251-265), Waggoner et al (Human Pathology,

1996, Vol. 27, pp. 494-502) and Haugland (Handbook of Fluorescent Probes and Research Chemicals, 1992, 5th Edition, pp. 235-269).

Claim 19 embodies the control cell of claim 16 wherein said membrane label is selected from the group consisting of long chain lipophillic indocarbocyanines, indocarbocyanines, indodicarbocyanines and analogs thereof, aminostyrl dyes, C18 rhodamine B, and C18 fluorescein dyes.

Waggoner et al teach the use of diL-C18 as a membrane label to establish the location of the cellular membrane during fluorescence imaging (Table 1, "Membrane location and fluidity"). Dil-C18 is defined as an analog of carboindocyanines as stated on page 25, line 1 of the instant specification.

Haugland teaches the octadecyl ester of fluorescein (page 253, under the heading "Lipophilic Fluorescein Probes"), the octadecyl indocarbocyanines and oxacarbocyanines and analogs thereof, dialkyamnostyrl and octadecyl rhodamine B (pages 260-261), all of which have the property of staining membranes.

Griwatz et al teach that the advantages of targeting intracytoplasmic proteins for labeling is that they are not cleaved by pericellular proteases, are not major immune response entities, and their expression is highly conserved and less variable (page 263, first column bridging paragraph to second column).

It would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made to substitute the membrane stains taught by Waggoner et al and Haugland for one or more of the antibodies which bind to distinct antigens on the surface of the cell. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Griwatz et al on the advantages of targeting cellular molecules which are not cleaved by proteases, wherein said targets are highly conserved and less variable than surface antigen-directed antibodies.

All claims are rejected.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Karen A. Canella whose telephone number is (571)272-0828. The examiner can normally be reached on 11 am to 10 pm, except Wed, Fri.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Larry Helms can be reached on (571)272-0832. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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